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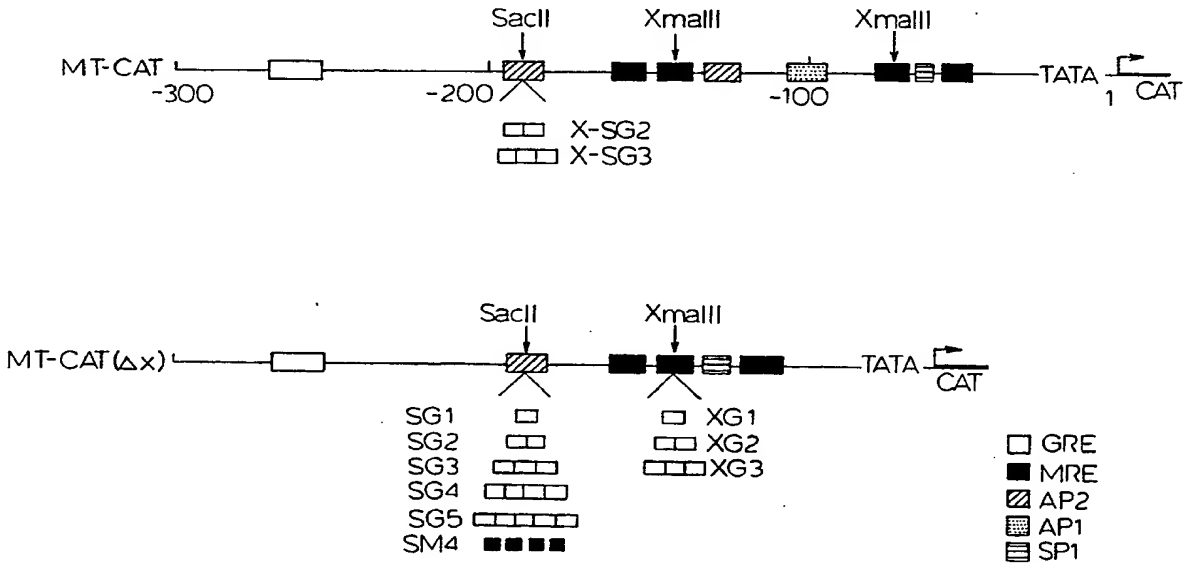

(57) Abstract

Synthetic inducible eukaryotic promoters for the regulation of transcription of a gene achieve improved levels of protein expression and lower basal levels of gene expression. Such promoters contain at least two different classes of inducible elements, usually by modification of a native promoter containing one of the inducible elements by inserting the other of the inducible elements. In embodiments, additional metal responsive elements (MREs) and/or glucocorticoid responsive elements (GREs) are provided to native promoters, particularly the hMT-IIA and MMTV-LTR promoters. One or more constitutive elements may be functionally disabled to provide the lower basal levels of gene expression.

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TITLE OF INVENTION

SYNTHETIC EUKARYOTIC PROMOTERS CONTAINING TWO INDUCIBLE ELEMENTS

FIELD OF INVENTION

The present invention relates to the generation of improved inducible mammalian expression systems.

BACKGROUND TO THE INVENTION

Mammalian expression systems are being widely used in the production, by recombinant techniques, of proteins that are extensively modified after translation. These systems can be either constitutive or inducible. It is advisable to use inducible systems for the expression of potentially cytotoxic proteins.

A key element in determining whether an expression system is constitutive or inducible is the promoter. Several mammalian promoters that can be induced in experimental systems have been characterized and promoters present in the metallothionein (MT) genes and in the mouse mammary tumour virus/long terminal repeat (MMTV-LTR) have been used extensively.

The best inducers for the MT promoter are heavy metal ions, such as cadmium (Cd) and zinc (Zn). The induction of the promoter is mediated by transcription factors which, after activation by metals, bind to the inducible metal responsive elements (MREs) that are present in the MT promoter. This promoter also contains several constitutive (non-inducible) elements that bind transcription factors which do not need to be activated and that are responsible for a basal level of gene expression. As a result of the presence of these constitutive elements, the non-induced level of expression of the MT promoter is significant and the induction ratio (the ratio between the inducible expression and the basal level of expression) is usually no greater than 5- to 10-fold. Attempts have been made to reduce the basal level of expression by removing some of the constitutive elements of the MT promoter. The

removal of these elements, however, also reduces the inducible level of expression.

The native human MT-IIA promoter, besides having the MREs and the constitutive elements, contains a single inducible glucocorticoid responsive element (GRE) and glucocorticoids, such as dexamethasone (dex), induce low levels of expression from the MT-IIA promoter in its native context.

The native MMTV-LTR promoter contains four inducible GREs and can be strongly induced by glucocorticoids. The basal level of expression is lower than that obtained with the human MT-IIA promoter but the absolute level of inducible expression is not as high.

Nucleic acid sequences, such as inducible elements, involved in the regulation of gene expression, may be located 5′ to, 3′ to, or within the regulated gene.

SUMMARY OF INVENTION

In accordance with the present invention, there is provided a synthetic inducible eukaryotic promoter for the regulation of transcription of a gene, comprising at least two different classes of inducible elements. Classes of inducible elements with which the invention is concerned include hormone-responsive elements (including GREs), metal-responsive elements (MREs), heat shock-responsive elements, interferon-responsive elements and cytokine responsive elements.

In one embodiment, the synthetic promoter provided herein is derived from a native promoter and one of the different classes of inducible elements is a native inducible element while another of the different classes of inducible elements is provided, such as by insertion into the native promoter or by activation of a normally-inactive element in the native promoter. While, in general two different classes of inducible elements are present in the novel synthetic promoter of the invention, combinations of three or more may be present, if desired.

The utilization of different classes of inducible elements in the synthetic promoters enables synergistic induction of a expression of a gene product in a eukaryotic expression system, particularly a mammalian expression system. That is, the level of gene expression obtained by induction of multiple classes of inducible element is greater than the sum of the individual gene expressions achieved by separate induction of the individual classes of inducible elements. In addition, overall levels of gene expression may be enhanced.

The synthetic promoters provided herein generally are derived from natural promoters by modification, as described in more detail herein, although such promoters also may be produced synthetically.

As mentioned above, inducible promoters may contain at least one constitutive element, which provides a basal level of gene expression in the absence of induction. In one embodiment of the invention, at least one constitutive element is functionally disabled, which generally results in a decreased level of basal gene expression and an increased ratio of induced gene expression to basal gene expression, when compared to the unmodified promoter. Such functional disablement of the at least one constitutive element may be effected by deletion from the native promoter and/or by insertion, for example, of an inducible element therein.

The present invention, therefore, provides, in preferred embodiments, improved inducible eukaryotic promoters containing not only native GREs and/or MREs but also additional GREs and/or MREs. Constitutive elements of native promoters may or may not be deleted in the improved promoters. The improved promoters may be synergistically induced when both a heavy metal ion and a glucocorticoid (such as dexamethasone) are used at the same time and both at least one MRE and at least one GRE are present. Synergistic induction results in levels of

gene expression that are much higher than those observed with unmodified promoters, such as the human MT-IIA or MMTV-LTR promoters. The new promoters also may contain fewer constitutive elements than unmodified promoters, which allows for a lower basal level of gene expression.

Conveniently the unmodified promoter may be the human MT-IIA or MMTV-LTR promoter. The responsive elements may conveniently contain the consensus sequence for such elements, for example,

5'-GATCTTGCGCCCGGCCCG-3' (SEQ ID NO: 2)

contains the MRE consensus sequence, and

5'-GATCTGGTACAGGATGTTCTAGCTACG-3' (SEQ ID NO: 1)

contains the GRE consensus sequence used in the embodiments of this invention.

Advantages of the present invention include:

a) high overall levels of gene expression,
b) decreased levels of basal gene expression,
c) synergistic induction of expression of a gene,
d) promoters customized with regard to induction ratio and/or responsiveness to convenient inducers.

BRIEF DESCRIPTION OF DRAWING

Figure 1 is a genetic map of the hMT-IIA promoter and of a modified promoter with various modifications effected to the hMT-IIA promoter in accordance with one embodiment of the present invention.

GENERAL DESCRIPTION OF INVENTION

As noted above, the novel promoter provided herein may be derived from a native promoter. In one preferred embodiment of the invention, the promoter contains at least one native inducible element which is an MRE and at least one different inducible element which is a hormone responsive element, particularly a glucocorticoid responsive element (GRE) provided in the native promoter by insertion.

Such an inserted GRE may be a synthetic molecule comprising a pair of complementary oligonucleotides containing the GRE consensus sequence. A plurality of GREs may be inserted into the native promoter in the form of a multimeric head-to-tail self-ligated element.

A particularly preferred embodiment of the invention provides a human metallothionein gene (hMT-IIA) promoter modified to contain at least one inducible GRE, so as to obtain a synergy of gene expression upon induction of the inducible MREs and GREs in a eukaryotic expression system, particularly a mammalian expression system, and preferably combined with an enhanced overall level of gene product expression. In this particularly preferred embodiment, multimeric head-to-tail GREs may be inserted into the native hMT-IIA promoter.

It is preferred also to disable at least one constitutive element of the native hMT-IIA promoter, such as by deletion of such element and/or by insertion of at least one GRE therein. In one illustrative Example, both deletion of constitutive elements and insertion of single or multiple GREs are employed to disable constitutive elements.

In another preferred embodiment of the invention, the promoter contains at least one native inducible element which is an HRE, particularly a glucocorticoid responsive element (GRE), and at least one different inducible element which is a MRE provided by insertion.

Such inserted MRE may be a synthetic molecule comprising a pair of complementary oligonucleotides containing the MRE consensus sequence. A plurality of MREs may be inserted into the native promoter in the form of a multimeric head-to-tail self-ligated element.

A particularly preferred embodiment of the invention provides a mouse mammary tumor virus/long terminal repeat (MMTV-LTR) promoter, modified to contain at least one inducible MRE, so as to obtain a synergy of gene

expression upon induction of the inducible GREs and MREs in a eukaryotic expression system, and preferably combined with an enhanced overall level of gene expression. In this particularly preferred embodiment, multimeric head-to-tail MREs may be inserted into the native MMTV-LTR promoter.

The novel synthetic inducible eukaryotic promoter provided herein may be incorporated into a vector for eukaryotic expression of a gene product, particularly when operatively connected to a gene to be expressed by the expression system. Such expression system may comprise eukaryotic cells containing the vector, particularly mammalian cells, such as Vero, CHO, HeLa, RatII fibroblasts and intestinal epithelial cells.

DESCRIPTION OF PREFERRED EMBODIMENT

In Figure 1, there are shown different versions of a new promoter incorporating various modifications in accordance with embodiments of the present invention. The new series of promoters are generated using the following methodology. A KspI DNA fragment containing 800 bp of the 5′ promoter region of the human MT-IIA gene (bases -740 to +60) was isolated from a plasmid containing the human MT-IIA gene (see Karin et al, (1982) Nature, 299, 797-802). After generating blunt ends, HindIII linkers were added and the fragment was inserted into pSVOATCAT, a plasmid containing the chloramphenicol acetyl transferase (CAT) gene used as a reporter gene, at the HindIII site 5′ to the CAT gene. Two constitutive elements (AP1 and AP2 - see upper map, Figure 1) of the original MT-IIA promoter were deleted by removing an XmaIII fragment (bases -79 to -129).

A pair of complementary oligonucleotides containing the GRE consensus sequence, a 5′ BamHI site and a 3′ BglII site was synthesized. The positive strand oligonucleotide sequence was:

5′-GATCTGGTACAGGATGTTCTAGCTACG-3′ (SEQ ID NO: 1)

Multimeric head-to-tail GREs were prepared by self-ligating the synthetic GRE oligonucleotide in the presence of BamHI and BglII. Single and multimeric GREs were inserted into the SacII site of the promoter (at base -175) or the XmaIII site of the promoter (at base -129) (see lower map in Figure 1). The insertion at the SacII element destroys a second AP2 site.

A pair of complementary oligonucleotides containing the MRE consensus sequence, a 5′ BamHI site and a 3′ BglII site was synthesized. The positive strand nucleotide sequence was:

5′-GATCTTGCGCCCGGCCCG-3′ (SEQ ID NO: 2)

Such oligonucleotides may be used to synthesize multimeric head-to-tail elements and single or multiple MREs may be inserted into the hMT-IIA promoter in an analogous manner to the GREs.

The MMTV-CAT vector for effecting similar GRE and/or MRE insertions to and optionally constitutive element deletions from the MMTV-LTR promoter was removed from plasmid p201 (Majors et al, (1981), Nature, 283, 253-258) using PstI and, after generation of blunt ends, inserted into the HindIII site of pSVOATCAT.

The new promoters were tested in transient CAT expression assays using RAT II fibroblasts, CHO (chinese hamster ovarian cells), VERO (monkey fibroblasts) and Hela (human cervical tumour cells) cells, expressing the glucocorticoid receptor. The results, reproduced in the Examples below, indicated that these new promoters generate very high levels of expression when cells normally expressing the glucocorticoid receptor or transfected with the glucocorticoid receptor gene are simultaneously induced with heavy metal ions and dexamethasone. The induced levels of expression obtained with these promoters are significantly higher than those observed with the wild-type human MT-IIA or MMTV-LTR promoters. At the same time the basal level of

expression was significantly lower than that observed with the wild-type human MT-IIA promoter.

EXAMPLES

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples. These examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitations.

Example 1

This Example illustrates the construction of modified hMT-IIA promoters containing additional GREs.

All MT expression vectors were derived from pSVOATCAT, a plasmid containing the chloramphenicol acetyl transferase (CAT) gene without any regulatory sequences (Gorman et al., Mol.Cell.Biol., 2, 1044, [1982]). MT-CAT, a control plasmid in which the CAT gene is under the regulation of the wild-type human MT-IIA promoter (hMT-IIA), was generated as described below. An 800 bp KspI fragment of the promoter region of the hMT-IIA (bases -740 to +60) (Fig. 1) was isolated. After generating blunt ends, HindIII linkers were added and the fragment was inserted into the HindIII site of pSVOATCAT, 5′ to the CAT gene. Plasmid MT-CAT-ΔX was generated by removing the XmaIII fragment (base -79 to -129) from the MT promoter of MT-CAT which contains the constitutive AP1-AP2 elements. To insert additional GREs, a pair of complementary oligonucleotides containing the GRE consensus sequence, a 5′ BamHI site and a 3′ BglII site were synthesized and multimeric head-to-tail elements were generated by self-ligating these synthetic sequences in the presence of BamHI and BglII. The positive strand

nucleotide sequence was SEQ ID NO: 1, as specified above. Monomeric or multimeric GREs then were inserted at either the SacII or the XmaIII site of the MT-CAT-ΔX vector after generation of blunt ends (Fig. 1). The number of GREs inserted was confirmed by DNA sequencing.

Example 2

This Example illustrates the use of an expression vector containing additional GREs.

The expression vector used in this example was SG2, which is a pSVOATCAT-derived CAT expression vector containing a modified MT-IIA promoter in which two additional GREs were inserted at the SacII site of MT-CAT-ΔX (Fig. 1). Fifteen μg of plasmid DNA were transfected into CHO cells using the calcium phosphate procedure (Graham et al (1973) Virology, 52, 456-467). After incubation for 5 hours at 37°C, the cells were shocked for 3 minutes with 15% glycerol in PBS. The monolayers then were incubated with the different inducers ($CdCl_2$ and/or dexamethasone) for 16 hours and cell extracts were prepared. The CAT activity then was measured using ^{14}C-Chloramphenicol as substrate and the radioactive acetylated product was extracted with xylene. Radioactive counts were determined in a scintillation counter.

In addition, the SG2 vector was compared with two other vectors that were constructed by inserting a wild-type MT-IIA promoter and the MMTV-LTR promoter into the HindIII site of the pSVOATCAT plasmid. Since CHO cells do not have glucocorticoid receptors, the cells were co-transfected with 10μg of a glucocorticoid receptor expression vector (Giguere et al, (1986) Cell, 46, 645-652). CAT expression assays were performed in quadruplicate and the standard deviation did not exceed 10%. Protein concentration was measured in each cell lysate and CAT activity was calculated for equivalent amounts of protein. The results from these experiments

are summarised in Table I below. (The Tables appear at the end of the descriptive text).

The results appearing in Table I show that the synergistic induction of the SG2 promoter with metals and dexamethasone generated a higher level of CAT gene expression than the wild-type MT-IIA and the MMTV-LTR promoters. At the same time, the induction ratio also was significantly improved.

Example 3

This Example further illustrates the use of a vector containing additional GREs.

Using a procedure similar to that of Example 1, the activity of the SG2 promoter was compared with that of the native MT-IIA promoter in VERO cells engineered to express glucocorticoid receptors (Giguere et al, (1986) Cell, 46, 645-652). In this Example, the cells also were co-transfected with an expression vector in which the ß-galactosidase gene was driven by a promoter, whose activity was not affected under the experimental conditions by heavy metals or glucocorticoids. After transfection and induction, an aliquot of the cell extract was used to measure the ß-galactosidase (ß-Gal) activity. This activity was used to standardize CAT activity measurements by taking into account the efficiency of transfection.

The results obtained are shown in Table II below, and it can be seen that they are very similar to those obtained with CHO cells (Table I) and demonstrate that dexamethasone acts synergistically with metal ions on the modified MT-IIA (SG2) promoter.

Example 4

This Example illustrates further modification to the expression vector and the results obtained.

Additional modifications were effected to the hMT-IIA promoter to introduce additional numbers of GREs and

multiple MREs at the SacII site and to introduce numbers of GREs at the XmaIII site, as detailed in Figure 1.

The resulting modified plasmid DNA was introduced into Vero cells as described in Example 3 and CAT gene expression was determined as described above. The results obtained are set forth in Table III below.

Example 5

This Example illustrates the construction and use of a modified MMTV-LTR promoter containing additional GREs.

Two MREs were inserted, using a similar procedure to previous examples, at the BfrI site of the MMTV-LTR promoter, which contains four GREs but has no MREs (Majors and Varmus, Nature 283: 253-258). Table IV shows that while the unmodified MMTV-LTR promoter was not inducible by Zn plus Cd, the modified promoter (BM2-MMTV) displayed a ten-fold induction. When BM2-MMTV was induced by dexamethasone plus Zn plus Cd a two-fold synergy in CAT expression was observed.

The results of the experiments represented in Examples 1 to 5 and Tables I to IV show that it is possible to achieve synergistic activation of transcription in the context of a modified hMT-IIA promoter by inserting additional inducible elements in the form of GREs and in the context of a modified MMTV promoter by inserting additional inducible elements in the form of MREs. Addition of the GREs to the hMT-IIA promoter and MREs to the MMTV promoter did not increase the basal level of reporter gene expression and the inducibility and transcriptional strength of the modified promoters were significantly improved over those of their wild-type counterpart. In contrast the exclusive insertion of four extra MREs (vector SM4) to the hMT-IIA promoter resulted only in a moderate improvement in MT promoter transcriptional strength and this improvement was accompanied by a significant increase in basal expression.

The unmodified hMT-IIA promoter in the MT-CAT vector could not be induced by dexamethasone in Vero cells transfected with the glucocorticoid receptor gene. However, the insertion of at least one additional GRE to the promoter was enough to confer glucocorticoid responsiveness and gene expression.

To analyze the impact of the number of additional GREs inserted and the site of insertion, two series of modified promoters were generated in the Examples by adding one or more GREs at either SacII site (SG series) or the XmaIII site (XG series) of MT-CAT-ΔX. All vectors were inducible by $CdCl_2$ and glucocorticoids. However, a minimum of two adjacent GREs was necessary to generate synergistic inducibility by simultaneous treatment of transfected Vero cells with $CdCl_2$ and dexamethasone, regardless of the site of insertion.

The induction ratio calculated for the modified hMT-IIA promoters was increased up to 6-fold as compared to the wild-type promoter. The fact that the insertion of additional GREs did not increase the basal level of gene expression in, for example, SG3 is an important factor in the improvement of this ratio. This observation emphasizes one of the advantages of generating synergistic transcription activation by adding different classes of inducible elements rather than constitutive ones, in accordance with the present invention.

SUMMARY OF DISCLOSURE

In summary of this disclosure, the inventors provide for the engineering and use of novel and improved inducible mammalian expression systems, in particular, the preparation and use of modified human MT-IIA promoters containing one or several additional glucocorticoid-responsive elements which can be synergistically induced by glucocorticoids and metal ions while maintaining a low level of basal gene expression. The induction ratio may be increased further by deleting

constitutive elements. A similar strategy may be used to generate improved mouse mammary tumour virus (MMTV) promoter by inserting additional metal-responsive elements. Modifications are possible within the scope of this invention.

TABLE I

Promoter	Inducer	CAT Activity (cpm)
MT-IIA	Control	5932
MT-IIA	100μM $ZnCl_2$ + 2μM $CdCl_2$	70235
MT-IIA	1μM Dexamethasone	3935
MT-IIA	100μM $ZnCl_2$ + 1μM Dexamethasone	70119 (12x)*
SG 2	Control	2893
SG 2	100μM $ZnCl_2$ + 2μM $CdCl_2$	22901
SG 2	1μM Dexamethasone	97068
SG 2	100μM $ZnCl_2$ + 2μM $CdCl_2$ + 1μM Dexamethasone	147713 (57x)*
MMTV-LTR	Control	751
MMTV-LTR	1μM Dexamethasone	20310 (27x)*

* = Induction Ratio

TABLE II

Promoter	Inducer	Standardised CAT Activity (U CAT/β-GAL)
MT-IIA	Control	19
MT-IIA	5μM $CdCl_2$	574
MT-IIA	1μM Dexamethasone	40
MT-IIA	5μM $CdCl_2$ + 1μM Dexamethasone	526 (27x)*
SG 2	Control	8
SG 2	5μM $CdCl_2$	114
SG 2	1μM Dexamethasone	230
SG 2	5μM $CdCl_2$ + 1μM Dexamethasone	1072 (134x)*

* = Induction Ratio

TABLE III

Promoter	Inducer	Relative CAT activity (% of MT-IIA control)
MT-IIA	Control	100
MT-IIA	5uM $CdCl_2$	1064
MT-IIA	1uM Dexamethasone	103
MT-IIA	5uM $CdCl_2$ + 1uM Dexamethasone	1074
SG1	Control	32
SG1	5uM $CdCl_2$	328
SG1	1uM Dexamethasone	957
SG1	5uM $CdCl_2$ + 1uM Dexamethasone	1364
SG2	Control	36
SG2	5uM $CdCl_2$	364
SG2	1uM Dexamethasone	1164
SG2	5uM $CdCl_2$ + 1uM Dexamethasone	2324
SG3	Control	50
SG3	5uM $CdCl_2$	596
SG3	1uM Dexamethasone	1821
SG3	5uM $CdCl_2$ + 1uM Dexamethasone	3156
SG4	Control	29
SG4	5uM $CdCl_2$	210
SG4	1uM Dexamethasone	386
SG4	5uM $CdCl_2$ + 1uM Dexamethasone	1317
SG5	Control	21
SG5	5uM $CdCl_2$	200
SG5	1uM Dexamethasone	136
SG5	5uM $CdCl_2$ + 1uM Dexamethasone	1117
XG1	Control	46
XG1	5uM $CdCl_2$	1755
XG1	1uM Dexamethasone	275
XG1	5uM $CdCl_2$ + 1uM Dexamethasone	1574
XG2	Control	12
XG2	5uM $CdCl_2$	519
XG2	1uM Dexamethasone	394
XG2	5uM $CdCl_2$ + 1uM Dexamethasone	1957
XG3	Control	11
XG3	5uM $CdCl_2$	107
XG3	1uM Dexamethasone	36
XG3	5uM $CdCl_2$ + 1uM Dexamethasone	229

TABLE III (CONTINUED)

Promoter	Inducer	Relative CAT activity (% of MT-IIA control)
X-SG2	Control	84
X-SG2	5uM $CdCl_2$	1482
X-SG2	1uM Dexamethasone	495
X-SG2	5uM $CdCl_2$ + 1uM Dexamethasone	2562
X-SG3	Control	146
X-SG3	5uM $CdCl_2$	1145
X-SG3	1uM Dexamethasone	833
X-SG3	5uM $CdCl_2$ + 1uM Dexamethasone	3383
SM4	Control	393
SM4	5uM $CdCl_2$	1485
SM4	1uM Dexamethasone	382
SM4	5uM $CdCl_2$ + 1uM Dexamethasone	1524

TABLE IV

Promoter	Inducer	Standardized CAT activity (CPM)
MMTV-LTR	control	1326
MMTV-LTR	Dex	135405
MMTV-LTR	Zn+Cd	225
MMTV-LTR	Zn+Cd+Dex	145416(102X)*
BM2-MMTV	control	1078
BM2-MMTV	Dex	92899
BM2-MMTV	Zn+Cd	10827
BM2-MMTV	Zn+Cd+Dex	196614(182X)*

* Induction ratio.

CLAIMS

What we claim is:

1. A synthetic inducible eukaryotic promoter for the regulation of transcription of a gene, comprising at least two different classes of inducible elements.

2. The promoter of claim 1 wherein said classes of inducible elements are selected from the group consisting of hormone-responsive elements (HREs), metal-responsive elements (MREs), heat shock-responsive elements (HSREs) and interferon-responsive elements (IREs).

3. The promoter of claim 2 which is derived from a native promoter and one of said different classes of inducible elements is a native inducible element and another of said different classes of inducible elements is a different inducible element provided in said native promoter.

4. The promoter of claim 3 wherein said different classes of inducible elements are selected to provide a synergistic level of expression of a gene product in a eukaryotic expression system.

5. The promoter of claim 1 wherein said different classes of inducible elements are selected to provide a synergistic level of expression of the gene operatively coupled thereto in a eukaryotic expression system.

6. The promoter of claim 2 wherein said different classes of inducible elements are selected to provide a synergistic level of expression of the gene operatively coupled thereto in a eukaryotic expression system.

7. The promoter of claim 1 which is derived from a native promoter, one of said different classes is a native inducible element and another of said different classes is a different inducible element provided in said native promoter, and wherein said different classes of inducible element are selected to provide a synergistic level of expression of the gene in a eukaryotic expression system.

8. The promoter of claim 1 which is derived from a native promoter containing at least one constitutive element and wherein said at least one constitutive element is functionally disabled.

9. The promoter of claim 8 wherein said at least one constitutive element is functionally disabled sufficient to provide a decreased level of basal gene expression and an increased ratio of induced gene expression to basal gene expression when compared to the native promoter.

10. The promoter of claim 8 wherein said at least one constitutive element is disabled by deletion from the native promoter and/or insertion of an inducible element therein.

11. The promoter of claim 3 wherein said at least one native inducible element is a metal-responsive element (MRE) and at least one said different inducible element is a hormone-responsive element (HRE).

12. The promoter of claim 11 wherein said at least one hormone-responsive element is at least one glucocorticoid-responsive element (GRE) and is provided in said native promoter by insertion.

13. The promoter of claim 12 wherein said inserted GRE is a synthetic molecule containing the GRE consensus sequence and having a positive strand having the nucleotide sequence:

5′-GATCTGGTACAGGATGTTCTAGCTACG-3′ (SEQ ID NO: 1)

14. The promoter of claim 13 wherein a plurality of GREs is inserted in said native promoter in the form of a multimeric head-to-tail element self-ligated in the presence of BamHI and BglII.

15. The promoter of claim 12 which is derived from a native promoter containing at least one constitutive element and wherein said at least one constitutive element is functionally disabled.

16. The promoter of claim 15 wherein said at least one constitutive element is disabled by deletion from the

native promoter and/or insertion of an inducible promoter therein.

17. The promoter of claim 16 wherein said at least one constitutive element is functionally disabled sufficient to provide a decreased level of basal gene expression and an increased ratio of induced gene expression.

18. The promoter of claim 17 wherein said native promoter is the hMT-IIA promoter.

19. The promoter of claim 12 wherein said native promoter is the hMT-IIA promoter.

20. The promoter of claim 19 wherein said insertion of said at least one GRE into the native promoter produces a synergy of gene expression upon induction of said at least one native MRE and at least one added GRE in a eukaryotic expression system.

21. The promoter of claim 20 wherein said insertion of said at least one GRE into the native promoter produces an enhanced level of gene expression in a eukaryotic expression system.

22. The promoter of claim 19 wherein multiple linked GREs are inserted into the native promoter.

23. The promoter of claim 19 wherein at least one native constitutive element is disabled.

24. The promoter of claim 23 wherein said constitutive element is disabled by deletion and/or insertion of at least one GRE therein.

25. The promoter of claim 24 wherein native constitutive elements AP1 and AP2 located between bases -79 to -129 of the native hMT-IIA promoter are deleted.

26. The promoter of claim 25 wherein at least one GRE sequence is inserted at the SacII site (base -175) of the native hMT-IIA promoter thereby disabling the AP2 constitutive element at that location.

27. The promoter of claim 19 wherein said at least one GRE is inserted at least one of the SacII site (base -

175) and the XmaIII site (base -129) of the native hMT-IIA promoter.

28. The promoter of claim 27 wherein two linked GRE sequences are inserted at the XmaIII site.

29. The promoter of claim 27 wherein three linked GRE sequences are inserted at the SacII site.

30. The promoter of claim 3 wherein said at least one native inducible element is a hormone-responsive element (HRE) and said at least one different inducible element is a metal-responsive element (MRE).

31. The promoter of claim 30 wherein said at least one hormone-responsive element is a glucocorticoid responsive element (GRE) and said MRE is provided in said native promoter by insertion.

32. The promoter of claim 31 wherein said inserted MRE is a synthetic molecule containing the MRE consensus sequence and having a positive strand having the nucleotide sequence:

5'-GATCTTGCGCCCGGCCCG-3' (SEQ ID NO: 2)

33. The promoter of claim 32 wherein a plurality of MREs is inserted into the native promoter in the form of a multimeric head-to-tail element self-ligated in the presence of BamHI and BglII.

34. The promoter of claim 31 wherein said native promoter is the MMTV-LTR promoter.

35. The promoter of claim 34 wherein at least two linked MREs are inserted into the native promoter.

36. A vector for eukaryotic expression of a gene product, comprising a synthetic inducible eukaryotic promoter comprised of at least two different classes of inducible elements.

37. The vector of claim 36 wherein said promoter is operatively connected to a gene.

38. The vector of claim 37 wherein said promoter is a modified native hMT-IIA promoter as claimed in claim 19.

39. The vector of claim 37 wherein said promoter is a modified native MMTV-LTR promoter as claimed in claim 34.

40. A eukaryotic expression system, comprising eukaryotic cells containing a vector as claimed in claim 37 for effecting induced gene expression.

41. The expression system of claim 40 wherein said eukaryotic cells are mammalian cells.

42. The expression system of claim 41 wherein said mammalian cells are selected from Vero, CHO, HeLa, RatII and epithelial cells.

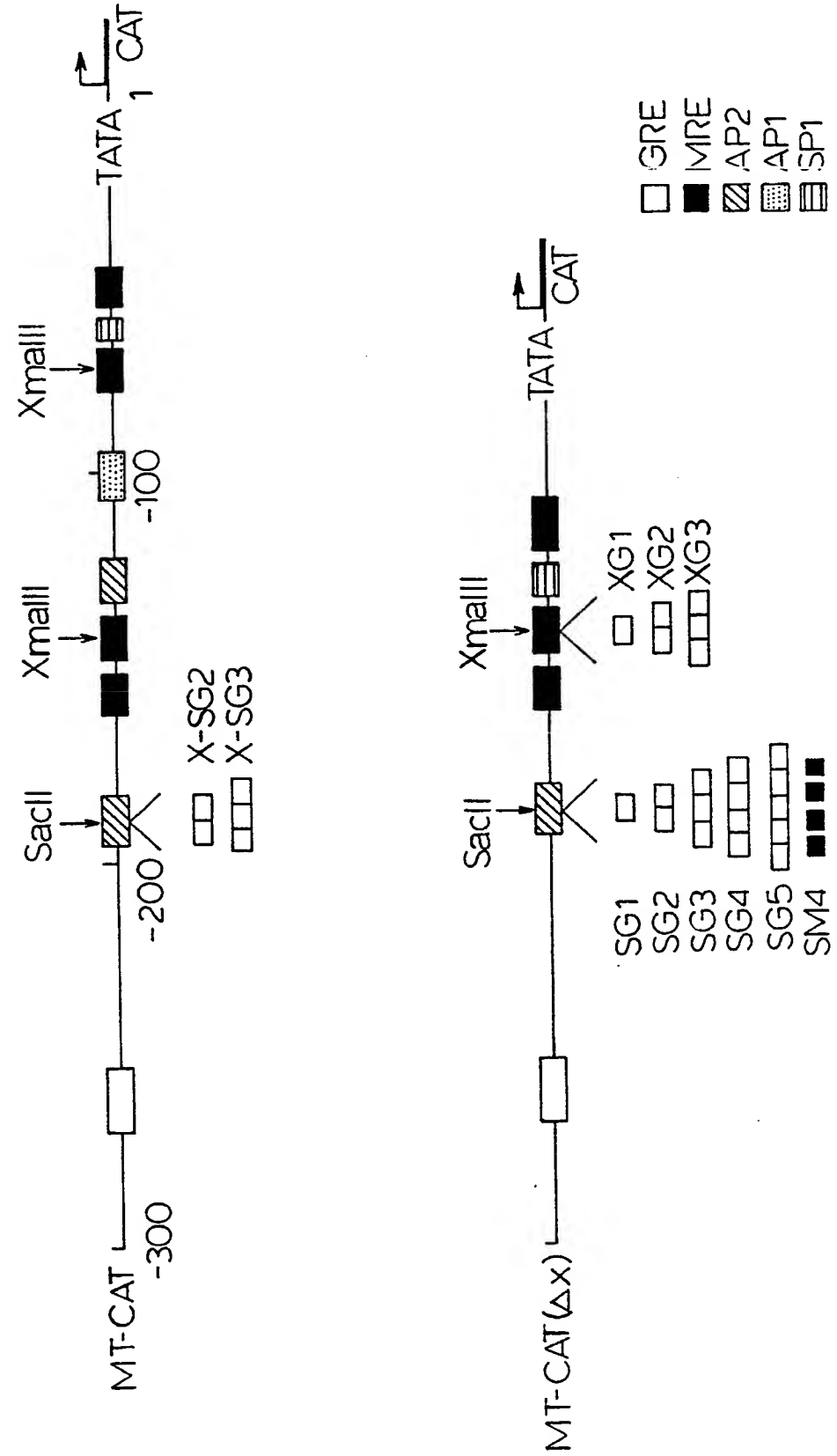

FIG.1.

INTERNATIONAL SEARCH REPORT

International Application No PCT/CA 93/00130

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N15/85; C12N5/10

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C12N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
P,X	NUCLEIC ACIDS RESEARCH. vol. 20, no. 11, 11 June 1992, ARLINGTON, VIRGINIA US pages 2755 - 2760 FILMUS, J. ET AL. 'Synergistic induction of promoters containing metal-and glucocorticoid-responsive elements' see the whole document ---	1-42
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 86, no. 10, May 1989, WASHINGTON US pages 3494 - 3498 3494-8 'Trans-activation by thyroid hormone receptors: functional paralleles with steroid hormone receptors' see page 3495, column 1, paragraph 2 4; figure 1 --- -/--	1,2,7, 36,37, 39-41

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
11 AUGUST 1993	27 -08- 1993
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	CHAMBONNET F.J.

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	WO,A,9 110 453 (THE UNITED STATES OF AMERICA, SECRETARY OF U.S.DEPARTEMENT OF COMMERCE) 25 July 1991 see figures 6,7 ---	1,36,37
X	WO,A,8 301 783 (UNIVERSITY PATENTS, INC.) 26 May 1983 see the whole document ---	1,2, 36-38
X	WO,A,9 112 258 (THE SALK INSTITUTE FOR BIOLOGICAL STUDIES) 22 August 1991 see claims 11,12 -----	1,2,36, 37

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

CA 9300130
SA 71873

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
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WO-A-9112258	22-08-91	AU-A-	7338391	03-09-91
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For more details about this annex : see Official Journal of the European Patent Office, No. 12/82